

NGF for the prevention of demyelination in the nervous system

The present invention concerns a process for prevention of demyelination in the nervous system by administering NGF. In addition, the invention concerns a pharmaceutical composition for treating diseases in which a demyelination of nerve fibers occurs as well as a process for its production.

The covering of nerve fibers in the nervous system (NS) with myelin is essential for the function of neuronal signal transmission. The myelin sheath is formed by oligodendrocytes (OL) in central nervous system (CNS) cells or Schwann cells which wrap these myelin sheaths around the axon of a nerve cell. Oligodendrocytes are part of a separate non-neuronal cell population distributed throughout the CNS as part of the neuroglia. These are cells that are essential for the regular function of neurons, in the case of oligodendrocytes by forming the myelin sheaths around the axons, thereby increasing electric conduction of nerve impulses (Kandel et al., Principles of Neural Science, 3rd Ed., Elsevier 1991, p. 22). Therefore the identification and characterization of factors which are responsible for preventing the demyelination is very important for the molecular understanding of demyelinating diseases and for the development of therapeutic agents.

It is well known that NGF acts on different subtypes of neuronal cells. In the periphery NGF is a survival factor for sensory and sympathetic neurons (Levi-Montalcini, R. and Angeletti, P. U. (1963) Dev. Biol. 7: 653-659; Chun, L. L. Y. and P. H. Patterson (1977) J. Cell Biol. 75: 694-704; Chun, L. L. Y. and P. H. Patterson (1977) J. Cell Biol. 75: 705-711; Crain, S. M. and E. R. Peterson (1974) Brain Res. 79: 145-152.), in the central nervous system NGF is a survival factor for cholinergic neurons and prevents the degeneration of cholinergic neurons in the basal forebrain (Gage et al. (1988) J. Comp. Neurol. 269: 147-155; Hefti et al. (1986) J. Neurosci. 6: 2155-2162; Hefti et al. (1986) J. Brain Res. 293: 305-311.).

With the results of Althaus et al. a new characteristic of NGF was identified (Althaus et al. (1992) Neurosci. Lett. 135: 219-223, and International Application No. WO 93/03140). NGF induced proliferation and differentiation of oligodendrocytes, a non-neuronal cell population. These oligodendrocytes are specialized and the only cells in the central nervous system which are capable of producing myelin and wrapping the myelin sheaths around the axons. The oligodendrocytes and myelin sheaths are susceptible to attack by auto-immune

processes, e.g., multiple sclerosis. Therefore, Althaus indicates that the induction of remyelination could be an important step in a therapeutic approach for multiple sclerosis.

In the present invention, another new characteristic of NGF has been discovered. NGF is able to prevent demyelination of nerve fibers of the nervous system of a mammal, preferably of a human being, by influencing the immune system or the blood brain barrier (endothelial cells, T cells, macrophages, monocytes and microglia cells) At the moment the mechanism by which NGF is effective is not yet clear. But prevention of demyelination is a new and unexpected activity of NGF. Preferably, the effective amount of NGF or active NGF fragments is between 10 and 300 pg NGF/ml CSF (cerebrospinal fluid).

Koliatsos et al. discloses that NGF prevents the degeneration of cholinergic neurons in the basal forebrain. This finding is exclusively focused on NGF-sensitive nerve cells (neurons) in a small area of the brain, whereas the present invention is directed to the prevention of demyelination throughout the brain.

This approach is completely unrelated to the known effect of NGF on cholinergic neurons or other neuronal populations.

The object according to the present invention is achieved by a process for preventing the demyelination of nerve fibers in the nervous system of a human being, wherein said human being is treated with an amount of nerve growth factor (NGF) or active fragments of NGF elective to prevent demyelination.

L. Massacesi et al. have developed and extensively characterized a novel model of experimental allergic encephalomyelitis (EAE) in a small non-human primate (Massacesi, L. et al., Ann. Neurol. 37 (1995) 518-530). In contrast to most forms of acute EAE in rodents and in other non-human primates, EAE in the common marmoset *Callithrix jacchus* (*C. jacchus*) is a clinically mild, relapsing-remitting disease characterized pathologically by early and prominent demyelination with astrogliosis that is highly reminiscent of human multiple sclerosis (MS). Thus, this unique laboratory model is most suitable for testing substances which are useful for the prevention of demyelination.

The early studies had the limited objective of exploring the feasibility of such treatment in an animal model that closely resembled human MS. In testing the present invention, acute demyelinating EAE was induced in marmosets by active immunization with 100 µg

recombinant rat myelin/oligodendrocyte glycoprotein (MOG) in adjuvant. Beginning 7 days after immunization, animals were treated with NGF or placebo (6 μ g/day) administered intracerebroventricularly by continuous infusion. Animals were monitored in a blinded fashion for clinical signs of EAE cerebrospinal fluid (CSF) pleocytosis (e.g., inflammation), and immune cellular and antibody responses, for a period of 28 days following immunization.

In the NGF-treated animals, clinical EAE was delayed and markedly suppressed in severity compared to the controls. Neuropathologic examination of the NGF-treated animals corroborated the observation of clinical protection; fewer and smaller perivascular inflammatory infiltrates accompanied only by minimal demyelination were seen in the treated animals. This was in contrast with the large perivascular foci of inflammation with extensive demyelination which are usually present at the acute phase of MOG-induced EAE and which were also observed in the control animals of this study. Surprisingly, the results have uncovered an unforeseen effect of NGF, namely protection against EAE. This effect could be mediated by interaction of NGF with the immune system of the periphery or via local mechanisms within the central nervous system, possibly suppression of inflammatory mediators such as cytokines or leukotrienes.

The term "NGF" or "active fragment of NGF" within the sense of the present invention refers to natural NGF, in particular mammalian NGF preferably natural human or murine NGF and all fragments or derivatives of NGF which have its desired biological activity, i.e., prevent the fiber demyelination of oligodendrocytes. Examples of NGF molecules which are suitable for the process according to the present invention are for instance NGF- β , NGF 2.5S or NGF 7S from the submaxillary gland of the mouse. These NGF molecules can, for example, be obtained commercially from Sigma (St. Louis, USA) or Boehringer Mannheim GmbH (Mannheim, DE). The process according to the present invention is preferably carried out with a human NGF, particularly preferably with human recombinant NGF- β . The production of an active NGF fragment by tryptic digestion of NGF is described by Mercanti et al. in Biochim. Biophys. Acta 496 (1977) 412-419. This fragment is composed of two linear oligopeptides which are linked by a disulfide bridge and contains the amino acid residues 10 to 25 and 75 to 88 of the amino acid sequence of NGF [according to the nomenclature of Angeletti and Bradshaw, Proc. Natl. Acad. Sci. USA 68 (1970) 2417-2421].

The present invention also concerns a pharmaceutical composition for the treatment of diseases in which a demyelination of nerve fibers occurs and which contains NGF or an

active fragment thereof as the active substance together with the usual pharmaceutical vehicles, auxiliary substances, fillers and diluents. The pharmaceutical composition preferably contains human NGF, especially human recombinant NGF- β . In addition, the composition can contain one or several pharmaceutically tolerated protease inhibitors, for example, aprotinin, preferably in a kit wherein NGF and said inhibitor are located in separate containers.

In order to produce pharmaceutical preparations, the composition according to the present invention can be processed with therapeutically acceptable vehicles. Suitable vehicles for the production of such solutions are water, polyols, sucrose, invert sugar and glucose. Suitable vehicles for injection solutions are water, alcohols, polyols, glycerol and vegetable oil.

In addition, the pharmaceutical preparations can contain preservatives, solvents, stabilizing agents, wetting agents, emulsifiers, salts for changing the osmotic pressure, buffers and, if desired, other therapeutic drugs.

Inflammatory toxic-metabolic or hypertoxic disorders may cause damage to the myelin sheaths. Examples of such disorders, or diseases, are:

Multiple Sclerosis:

- classical (Charcot type)
- acute multiple sclerosis (Marburg type)
- diffuse sclerosis (Schilder)
- neurooptic myelitis (Devic)
- concentrical sclerosis (Baló)

**ADEM (acute disseminated encephalomyelitis
and perivenous encephalomyelitis):**

- (post- and parainfectious, post-vaccinal or "spontaneous")

Demyelination caused by virus:

- subacute sclerosing panencephalitis (SSPE)
- progressive multifocal leucoencephalopathy (PML)

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- AIDS-encephalopathy and -myelopathy
- tropical paralysis (HTLV I)

Demyelination caused by toxic metabolism:

- central pontine myelinolysis
- Marchiafava-Bignami syndrome
- funicular myelosis (vitamin B12 deficiency)

Demyelination caused by hypoxia/ischemia:

- subcortical arteriosclerotic encephalopathy (Binswanger's disease)
- post-hypoxic leucoencephalopathy

The Guillain-Barré syndrome (B. Vinken, Handbook of Clinical Neurology 7, Diseases of Nerves, Part I, Chapter 19 (1970) pp. 495 et seq.) is the most frequently observed type of peripheral polyneuritis.

Demyelination usually occurs subacutely, or even acutely, whereas slow development taking place over a number of weeks, or even months, is rarely observed. Hence, it is important that when polyneuritis has been diagnosed, treatment with NGF should be initiated immediately so as to prevent demyelination, as is described in the present invention. At the very onset of polyneuritis, as in Multiple Sclerosis, an inflammation of the optic nerve is usually observed. Thus, the present invention is also directed to the treatment of inflammatory diseases of the optic nerve. The action of NGF on the optic nerve is shown in Fig. 3.

Diseases in which a demyelination of nerve fibers occurs and which can be treated with the aid of the pharmaceutical composition according to the present invention can, for example, be caused by inflammation, autoimmune processes, enzymes or toxins. Examples of such diseases are, for instance, Multiple Sclerosis, slow virus encephalitis, various forms of myelitis or heavy metal poisoning. According to the invention it is preferred to administer NGF immediately after an inflammatory disease (causing further a demyelination) of an optic nerve is recognized.

The composition according to the present invention is preferably administered systemically. The administration can be carried out by methods familiar to a person skilled in the art, for

example, intrathecally, intravenously or subcutaneously. For the intrathecally or intravenous administration, NGF can be dissolved, for example, in physiological saline.

The addition of protease inhibitors, e.g., aprotinin, is not absolutely necessary when NGF is administered daily but does afford protection against proteases which could inactivate NGF. The preferred lower limit for the daily administered NGF dose is at a concentration between 0.05 µg and 5 µg/kg body weight. The administration of NGF is preferably carried out over a longer time period, i.e., longer than a day, preferably at least 48 hours.

The following examples, references and the figures are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

Brief Description of the Figures:

Figure 1a shows the experimental procedures for preventing acute EAE according to the present invention.

Figure 1b shows the clinical course of experimental autoimmune encephalomyelitis in placebo (Cytochrome C, n=2) and NGF (n=2) treated animals. The NGF treated animals showed a significant amelioration of the clinical score versus placebo treated animals.

Figure 1c shows the EAE (experimental allergic encephalomyelitis) reaction in four different marmosets. Marmosets 26.33.93 and 26.17.94 are treated on day 7 after immunization with placebo (cytochrome) whereas marmosets 26.29.94 and 26.30.92 are treated on day 7 after immunization with NGF.

Figure 2 is a photomicrograph of frontal sections through the brains of marmosets (Luxol Fast Blue Staining), depicting two representative areas from the prosencephalon and mesencephalon. The number of lesions (arrows point to examples in the photomicrographs) is lower in the NGF treated animal than in the marmoset receiving placebo-infusion of cytochrome C (magnification: 4x).

Figure 3

is a higher magnification of cross sections through the optic nerves of marmosets (Luxol Fast Blue-staining). Note the severe inflammation and demyelination in a cytochrome C-treated EAE-animal, in contrast to the absence of lesions in the marmoset receiving intraventricular NGF-infusion. Magnification: ca. 100X.

Preparation of Animals**1. Placement of intracerebroventricular catheters**

A technique for implanting indwelling cannulas in the brain ventricles of marmosets has been developed. Seven days prior to immunization for EAE, surgery is carried out after the animal is anaesthetized with ketamine/midazolam (20 mg/kg). The skin above the skull is shaved and surgically disinfected with surgical scrub and ethyl alcohol. The animal is positioned in a stereotaxic table on a K-pad and a sterile field is created over the skull. A skin incision is made and the skull exposed. The dura meninges are exposed through a small hole drilled in the bone of the skull, in regard to the appropriate coordinates for the right lateral ventricle (according to a published atlas of marmoset brain anatomy). A 25 gauge 5 mm-long stainless steel guide containing a 35 gauge polycarbonate cannula is then inserted in the cerebral ventricle, and secured to the bone with 2 lateral screws and a thin layer of dental cement. A second skin incision is made in the right flank of the animal. A 25 gauge polyvinyl catheter is connected with the ventricular cannula and then tunneled under the skin to the flank incision. At this point the skull incision is closed with sutures and the flank end of the polyvinyl catheter is connected to the flow regulator of a mini osmotic Alza pump filled with saline. The flank incision is then closed and the animal monitored for recovery. Although no significant risk of infection has been reported using this technique in rodents and higher mammals, Clamoxyl at 10 mg/kg BID per os is administered as a systematic prophylactic procedure against secondary infection of the central nervous system.

2. Replacement of osmotic minipumps

For pump replacement, the animal is first sedated (ketamine/midazolam), the hair on one flank trimmed and the skin disinfected using surgical, sterile technique. The animal is placed on a K-pad to prevent hypothermia. A small skin incision (0.5 cm) is made on the flank and the pump connected with the intracerebroventrical catheter and inserted in the subcutaneous space, then the incision is closed with surgical staples. Although no infectious complication

has been observed using this technique, Clamoxyl at 10 mg/kg intramuscularly x 1 is administered prophylactically at the time of pump insertion. The size of the pump suitable for marmosets is the 200 μ l capacity model 2002, i.e. the smallest available.

Example 1

Prevention of acute *C. jacchus* EAE with NGF

1. Immunizations

EAE is induced with 100 μ g of rat recombinant myelin/oligodendrocyte glycoprotein (MOG) in complete Freund's adjuvant followed by intravenous administration of 10^{10} killed Bordetella Pertussis organisms on the day of immunization and again 48 hours later. In preparation for immunization, antigen and adjuvant are emulsified under sterile conditions. The animal is then anesthetized with ketamine/midazolam and 100 μ l of the mixture is injected intradermally into four sites in the shoulder and hip areas. Prior to injection the sites are shaved, cleaned three times with surgical scrub and then twice with ethyl alcohol. Bordetella Pertussis is injected slowly (over 5 minutes) after placement of a 21 gauge catheter in the popliteal vein using the same skin preparation technique. A second Bordetella Pertussis injection is given 48 hours later using the same technique.

2. Treatments

Beginning on day 7 after immunization (before the appearance of clinical signs) the animals receive either placebo (cytochrome) or NGF delivered via a cannula implanted in the lateral ventricle and connected by a mini catheter to an osmotic Alza mini pump implanted under the skin of the flank; the pump containing NGF or placebo is implanted on day 0 (to account for the dead volume of the mini catheter) and delivers $6 \pm 1 \mu$ l/day until day 28. The dose of NGF is 6 μ g/day, determined on the basis of preliminary experiments in marmosets. Treatment is continued for a total of 21 days and the animals are euthanized (Figure 1a and table 2).

3. Observation of Clinical Course

Signs of EAE are monitored daily by blinded observers. Proliferative responses to myelin antigens are measured in blood on days 0, 14 and 28 after immunization. The CSF

inflammatory responses and the CSF concentrations of NGF are monitored at days 0, 14 and 28 after immunization.

4. Histologic examination

Neuropathologic examination of the brain and spinal cord is performed according to standard published techniques. Animals are euthanized at the end of the 28 day period and the nervous system perfused. Under deep pentobarbital anesthesia a thoracotomy is performed and a 14 gauge catheter is introduced and secured in the left ventricle. The right atrium is then opened and 200 ml of cold phosphate buffered saline are perfused through the heart. The descending aorta is then clamped in order to preserve spleen, inguinal lymph nodes, and the lower portion of the spinal cord as a supply of fresh or cryopreserved tissues for immunologic studies. Two hundred ml of a 2.5% solution of glutaraldehyde in phosphate buffer, pH 7.4 are then perfused as fixative. Brain hemispheres and spinal cord are dissected and after further fixation are prepared for histopathologic analysis according to our standard protocol. Eight 2.5 mm thick sections are cut in a plane perpendicular to the intercallosal line and embedded in paraffin for cutting and routine stains (hematoxylin/eosin and luxol fast blue). Some tissue is saved for processing thin (plastic) sections and electron microscopy, in order to obtain an ultrastructural analysis of myelin. Immunohistochemistry is performed on fixed tissues or on cryopreserved specimens from the caudal spinal cord, including staining with anti-MOG, anti-PLP and anti-MAG antibodies. In addition to ultrastructural analysis, these studies provide a valid assessment of the remyelinating process, if any, in and around the inflammatory lesions.

5. Cytokine gene expression in the central nervous system

These studies are carried out on fresh frozen sections of the spinal cord. Semi-quantitation of tumor necrosis factor (TNF- α), lymphotoxin (TNF- β), IL-2, IL-6, IL-10 and transforming growth factor- (TGF- β) are performed according to the protocol in use at the UCSF laboratory. Previous work has already established that the proinflammatory cytokine TNF- α likely plays a major role in promoting inflammation and demyelination in marmoset EAE. This proposed approach acts as a preliminary screen to determine whether the NGF-induced protection against EAE is mediated via modulation of local cytokines in the nervous system.

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6. Treatment groups

There are two experimental groups used:

- a) NGF, 6 µg/day intracerebroventricularly
- b) Placebo (Cytochrome c), 6 g/day intracerebroventricularly

Treatments begin 7 days after immunization and are continued until day 28 after immunization.

List of References

- Althaus et al., Neurosci. Lett. 135 (1992) 219-223
Angeletti and Bradshaw, Proc. Natl. Acad. Sci. USA 68 (1970) 2417-2421
Chun, L. L.Y., and Patterson, P. H., J. Cell Biol. 75 (1977) 694-704
Chun, L. L.Y., and Patterson, P. H., J. Cell Biol. 75 (1977) 705-711
Crain, S. M., and Peterson, E. R., Brain Res. 79 (1974) 145-152
Gage et al., J. Comp. Neurol. 269 (1988) 147-155
Hefti et al., J. Brain Res. 293 (1986) 305-311
Hefti et al., J. Neurosci. 6 (1986) 2155-2162
International Application No. WO 93/03140
Kandel et al., Principles of Neural Science, 3rd Ed., Elsevier 1991, p. 22
Levi-Montalcini, R., and Angeletti, P. U., Dev. Biol. 7 (1963) 653-659
Massacesi, L. et al., Ann. Neurol. 37 (1995) 518-530
Vinken, B., Handbook of Clinical Neurology 7, Diseases of Nerves, Part I, Chapter 19 (1970)
pp. 495 et seq.

Patent Claims

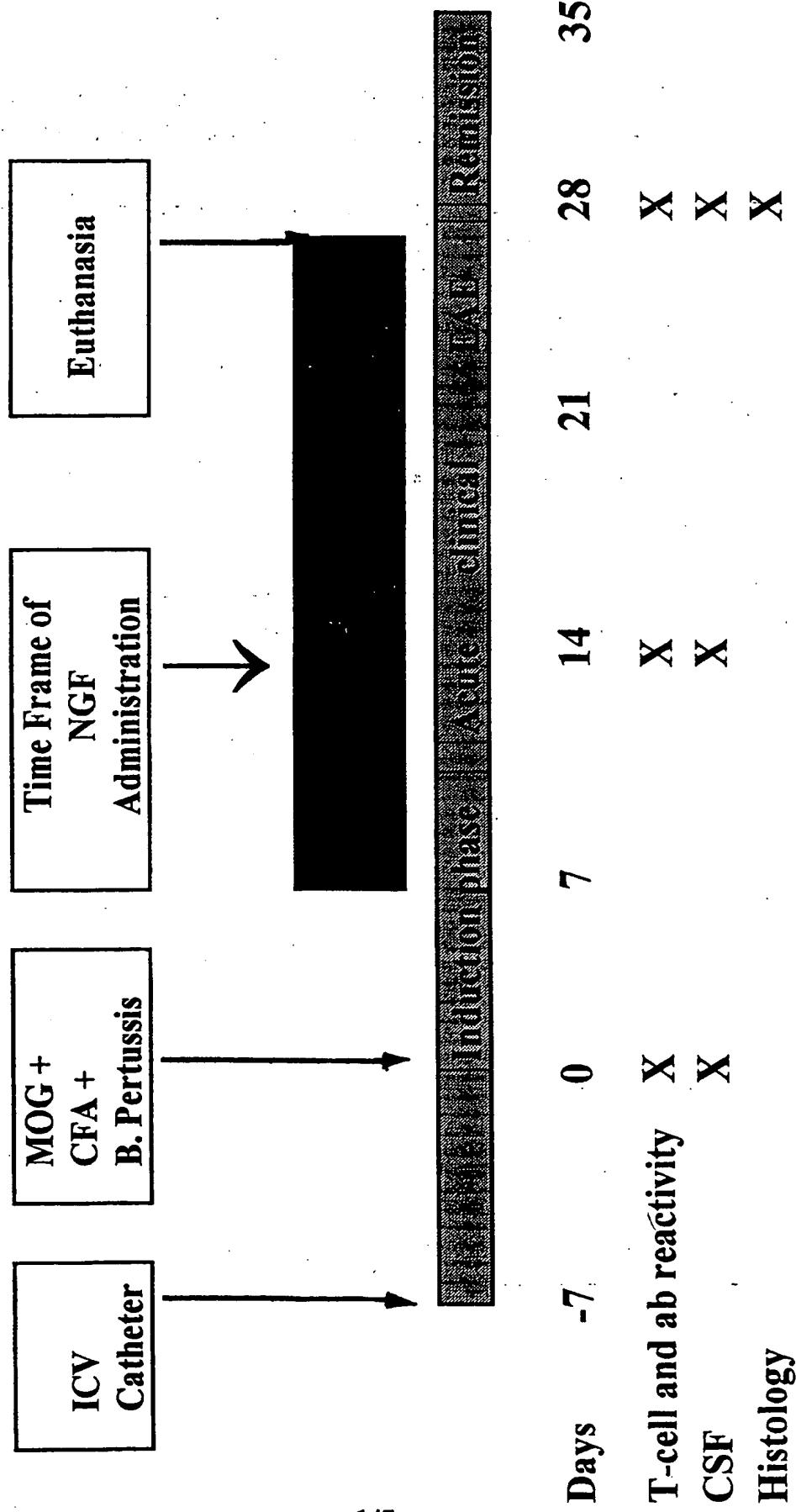
1. A process for preventing the demyelination of nerve fibers in the nervous system of a human being wherein said human being is treated with an amount of nerve growth factor (NGF) or active fragments of NGF effective to prevent demyelination.
2. The process according to claim 1, wherein said fragments are selected from the group consisting of NGF 2.5S and NGF 7S.
3. The process according to claim 1, wherein said nerve growth factor is human recombinant NGF- β .
4. The process according to claim 1, further comprising the administration of at least one protease inhibitor in combination with said NGF.
5. The process according to claim 4, wherein said protease inhibitor is aprotinin.
6. The method according to claim 1, wherein said NGF is administered to a patient in need of such treatment and said NGF is administered in an amount sufficient to produce a concentration of NGF or an active fragment of NGF between 0.05 μ g and 5 μ g/kg body weight.
7. A pharmaceutical composition for the treatment of diseases in which demyelination of nerve fibers occurs, comprising NGF or an active fragment thereof in combination with a pharmaceutical vehicle, auxiliary substance, filler or diluent.
8. The composition according to claim 7, wherein said fragment is selected from the group consisting of NGF 2.5S and NGF 7S.
9. The composition according to claim 7, wherein said NGF is human recombinant NGF- β .
10. The composition according to claim 7, further comprising at least one protease inhibitor in an amount sufficient to prevent inactivation of NGF by proteases.
11. The composition according to claim 10, wherein said protease inhibitor is aprotinin.

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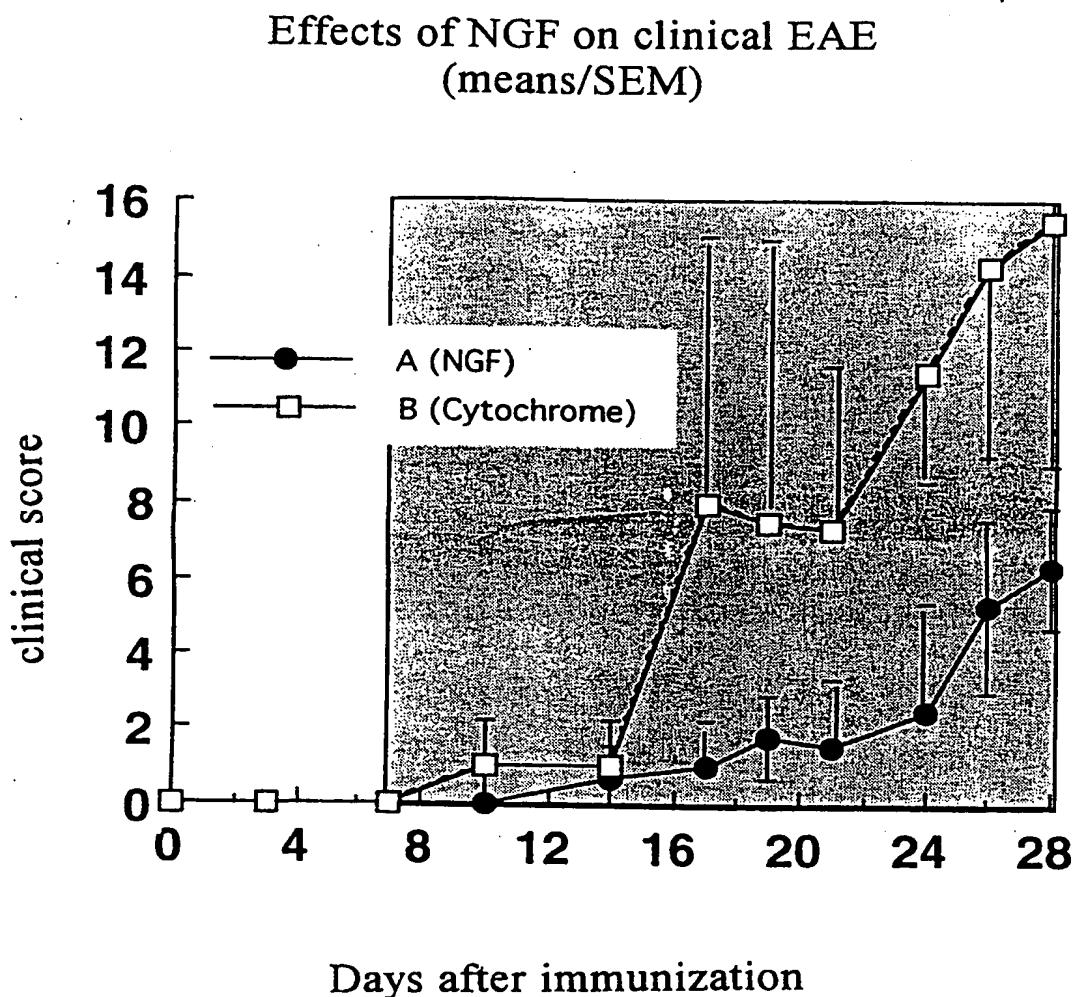
12. A process for preventing further demyelination in a patient having a disease in which a demyelination of nerve fibers occurs, comprising administering an amount of nerve growth factor or an active fragment thereof effective to prevent further demyelination.
13. The process according to claim 12, wherein the nerve growth factor is administered intravenously or intrathecally.
14. A method for preventing further demyelination in a patient having an inflammatory disease of the optic nerve, comprising administering an effective amount of NGF or an active fragment of NGF selected from the group consisting of NGF 2.5S and NGF 7S.
15. The method according to claim 14, wherein said effective amount of NGF or an active fragment of NGF is between 10-300 pg NGF/ml blood.
16. A kit for preventing further demyelination of nerve fibers, comprising NGF and at least one protease inhibitor in separate containers.

Fig. 1a

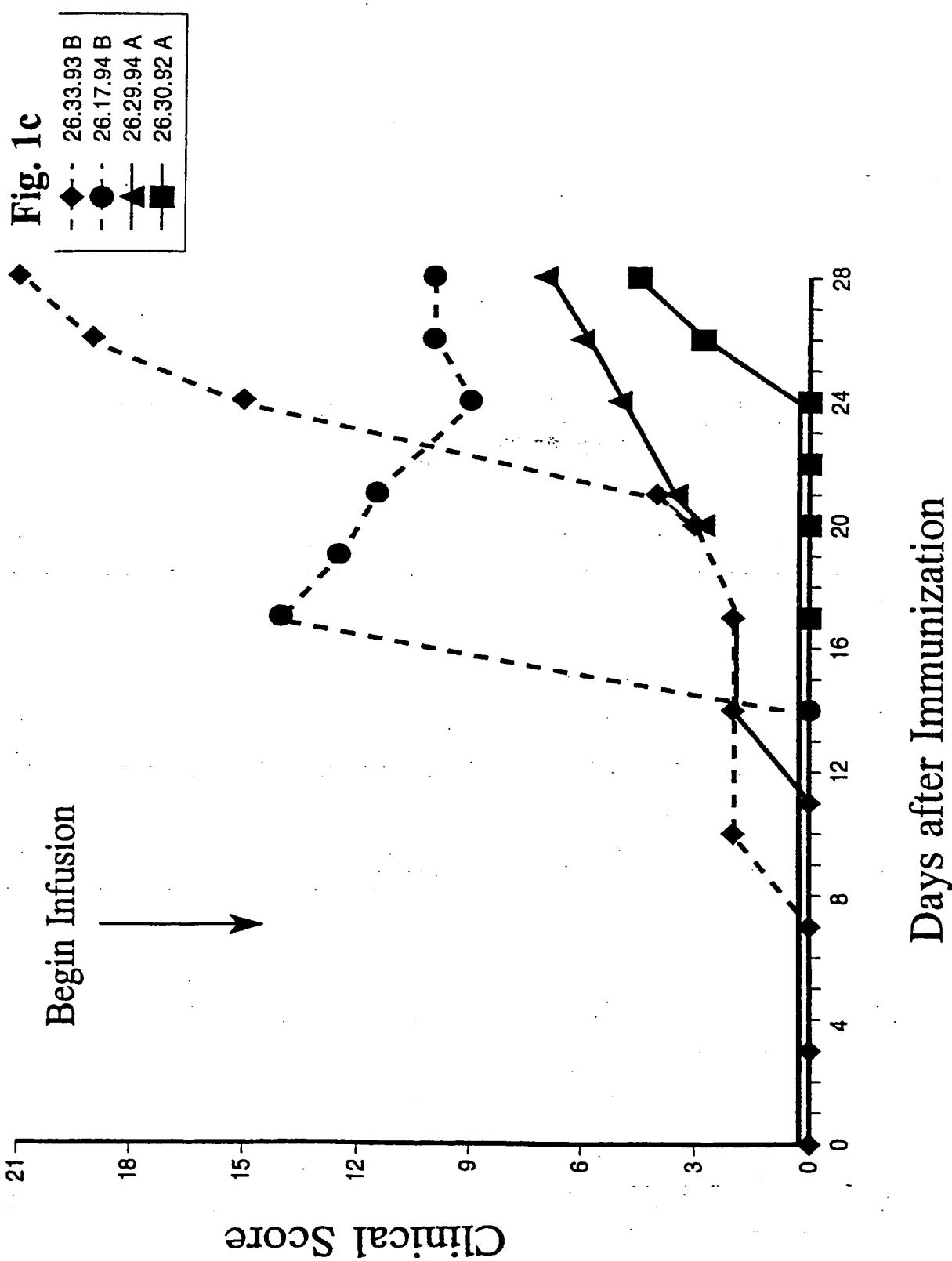
Prevention of acute EAE



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Fig. 1b

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Fig. 2

NGF
(animal-no. 26.30.92)

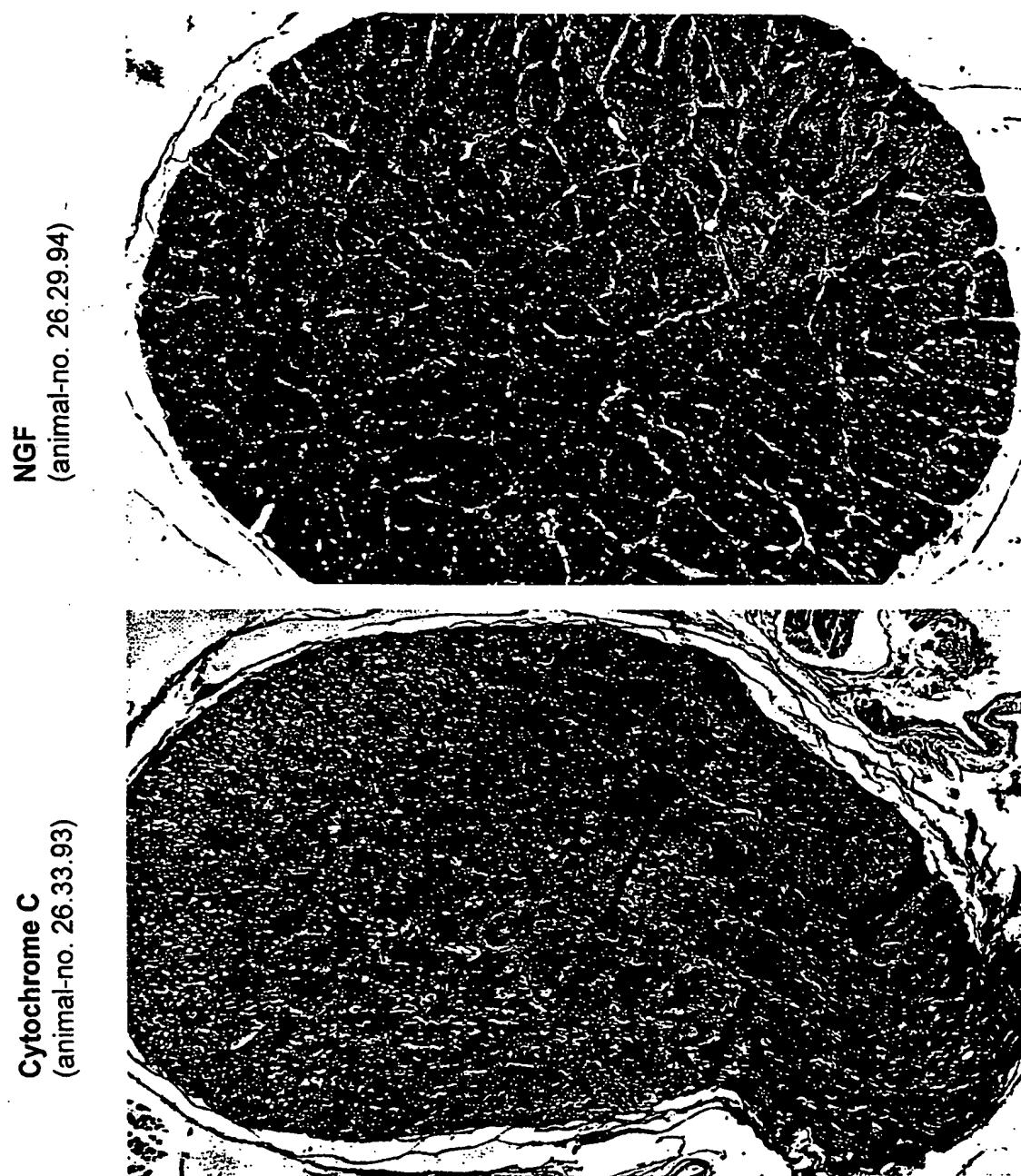


Cytochrome C
(animal-no. 26.17.94)



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Fig. 3



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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/02029

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/18 A61K38/55 // (A61K38/55, 38:18)

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 03140 A (MAX-PLANCK GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN) 18 February 1993	1-16
Y	cited in the application see page 3, line 27-32 see page 4, line 12-19 & DE 41 25 933 A (MAX-PLANCK GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN EV) 11 February 1993	1-16
X	EP 0 731 108 A (BOEHRINGER MANNHEIM GMBH) 11 September 1996	1, 3, 6, 7, 9, 12, 13
Y	see column 2, line 1-24	1-16
P, X	WO 97 17087 A (GENENTECH INC) 15 May 1997 see page 4, line 14-22	7-9



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 98/02029

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
WO 9303140	A	18-02-1993	DE	4125933 A		11-02-1993
EP 0731108	A	11-09-1996	AU	5103696 A		02-10-1996
			CA	2214999 A		19-09-1996
			WO	9628180 A		19-09-1996
			EP	0814828 A		07-01-1998
			JP	10504320 T		28-04-1998
WO 9717087	A	15-05-1997	AU	7518696 A		29-05-1997